

Morphometry of the masseter muscle and topographic location of the masseteric nerve: anatomical study in terms of BTX-A and facial reanimation applications

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ABSTRACT

Aims: The aim of this study was to investigate the morphometry of the masseter muscle (MM) and the topography of the masseteric nerve (MN) innervating the MM.

Methods: The MM and MN were examined on 18 sides (female: 4, male: 5) of formaldehyde-fixed adult cadavers in the laboratory of Kocaeli University Faculty of Medicine, Department of Anatomy. The MM and its surroundings were exposed by dissection. The morphometric measurements of the MM were obtained using a digital caliper. Tragus and lateral canthus landmarks were used for the location of the motor nerve of the MM.

Results: In this study, morphometric measurements of the MM were presented. A statistically significant difference was found between sexes in morphometric measurements related to the height and width of the muscle ($p < 0.05$). MM thickness was measured as 6.47 (6.06-6.50) mm. The median value of the distance between the tragus and lateral canthus was 76.04 (72.36-79.02) mm. Accordingly, the branching point of the MN; the vertical distance from the midpoint of the distance between the tragus and lateral canthus to the nerve was 42.72 (39.09-44.50) mm.

Conclusion: We believe that determining the topographic location of the MN using standard anatomical landmarks will make important contributions to both facial reanimation and BTX-A applications.

Keywords: Masseter muscle, masseteric nerve, facial reanimation, botulinum toxin type A, masseter muscle hypertrophy

INTRODUCTION

One of the clinically important problems associated with the masseter muscle (MM) is masseteric muscle hypertrophy (MMH).^{1,2} Botulinum toxin type A (BTX-A) application stands out as a safer and more effective option compared to invasive methods such as partial surgical resection and osteotomy for the treatment of this condition, which may cause aesthetic concerns.^{3,4} BTX-A reduces muscle activity by creating a transient synaptic blockade at the neuromuscular junction. However, the contractile function of the muscle begins to gradually return within a few weeks depending on the cellular regeneration process and although this process shows individual differences, it usually ends with the muscle reaching its pretreatment strength in approximately 6 months.¹ In order to perform BTX-A application safely and effectively, it is of great importance to know the anatomical structure of the MM and the location of the masseteric nerve (MN), the nerve innervating this muscle, in detail.

On the other hand, facial paralysis is a complex disease with variable etiology and severity and may lead to both physical

and psychological complications.^{5,6} In the treatment of this condition, various surgical options are available for facial reanimation. However, timing is critical in these operations because intervention should be performed within a certain period of time in order to connect the damaged facial nerve to another intact nerve.⁵ The potential role of the MN in facial rehabilitation was first described in 1978 and its use in facial reanimation became widespread in the following years.⁷⁻⁹ MN transfer is surgically advantageous due to its low morbidity rate and close location to the facial nerve. In addition, faster recovery compared to contralateral facial nerve graft makes this method attractive. However, despite all these advantages, surgeons report that dissection of this nerve is technically challenging.¹⁰ Therefore, the identification of reliable and reproducible anatomical landmarks that will facilitate the surgical detection of the MN may significantly help surgeons.

The MN is increasingly used in facial reanimation through three primary approaches: direct motor neurotization, babysitter and double innervation techniques, and the

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innervation of neuromuscular transplants. In direct motor neurotization, the MN is directly coapted to the facial nerve branches. Its location within the “subzygomatic triangle,” formed by the zygomatic arch, anterior border of the temporomandibular joint, and frontal branch of the facial nerve, allows for precise and efficient identification. In babysitter procedures, the MN temporarily innervates the facial nerve during long reinnervation periods to prevent muscle atrophy, and in some cases, it remains as a permanent coaptation to enhance facial movements. For neuromuscular free tissue transfers, the MN is commonly used to power free muscle grafts, such as gracilis muscle transfers, for smile reanimation, often leading to quicker functional recovery, with initial movement observed within 2–4 months.¹¹

In the literature, studies on the MN, the motor nerve of the MM, are limited compared to other cranial nerves and the anatomical, topographic and functional data on this nerve are not comprehensive enough. This study aims to examine the detailed anatomical structure and morphometric parameters of the MM and to define the topographic localization of the motor point of the muscle with reliable and reproducible anatomical landmarks that are highly usable in surgical procedures. The findings obtained in this direction are expected to provide intraoperative guidance in surgical applications and to increase surgical success by reducing complications in neurological rehabilitation and nerve transfer procedures.

METHODS

Ethics

Approval for this study was obtained from Kocaeli University Faculty of Medicine Non-interventional Clinical Researches Ethics Committee (Date: 19.12.2024, Decision No: GOKAEK 2024-/21.01). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. In this study, the MM was examined on 4 female and 5 male (total 18 sides) fixed cadavers. Cadavers with previous deformations or surgical procedures in the examined area were not included in the study.

Dissection Stages

Skin, superficial musculoaponeurotic tissue and adipose tissue were removed. The MM and surrounding structures were preserved and dissected carefully. Measurements were taken to determine the morphometric properties of the MM (Figure 1). The thickness of the MM was measured at the most bulging point of the muscle. Digital caliper was used for measurements.

Defined measurement points:

A: The highest point of the anterior edge of the superficial part of the MM

B: The highest point of the posterior edge of the superficial part of the MM

C: The highest point of the posterior edge of the deep part of the MM

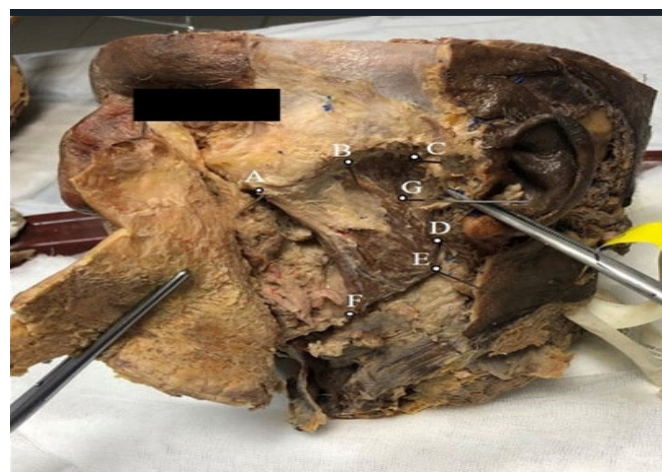


Figure 1. Measurement points used for the MM

D: The lowest point of the posterior edge of the superficial part of the MM

E: The gonion point

F: The lowest point of the anterior edge of the superficial part of the MM

G: The lowest edge of the deep part of the MM

Morphometric measurements related to the MM:

A-B: Upper edge length of the superficial part of the MM

A-F: Anterior edge length of the superficial part of the MM

B-C: Superficial upper edge of the deep part of the MM

B-D: Posterior edge length of the superficial part of the MM

B-G: Superficial anterior edge of the deep part of the MM

C-G: Posterior edge length of the deep part of the MM

E-D: Lower posterior edge length of the superficial part of the MM

F-E: Lower anterior edge length of the superficial part of the MM

To expose the MN, the MM was freed from its origin under the zygomatic arch and its insertion at the masseteric tuberosity. The muscle was reflexed from back to front and the branching point of the MN and the motor nerve entering the superficial and middle part of the muscle were identified (Figure 2). The branching point of the nerve was marked on the skin. Tragus and lateral canthus landmarks were determined on the skin to determine the entry point of the nerve into the muscle. The distance between the tragus and the lateral canthus (a) and the vertical distance from the midpoint of this line to the branching point of the MN were measured with a digital caliper (Figure 3).

Statistical Analysis

The data analyses of the study were performed with IBM SPSS Statistics 25.0 (IBM Corp. Armonk, New York, USA). Descriptive statistics of the variables in the study are given

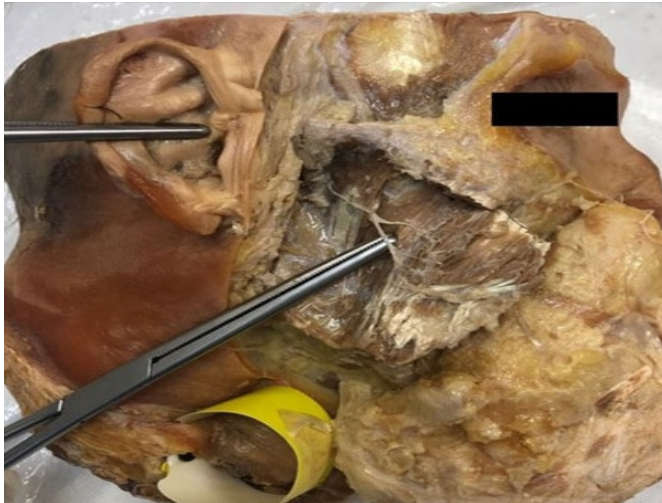


Figure 2. Branching point of the MN



Figure 3. Topographic location of the MN (a. Midpoint of the distance between the tragus and lateral canthus, b. Branching point of the MN)

as number of units, median and quartile values. Mann-Whitney-U test was used for comparisons between sexes and Wilcoxon test was used for comparisons between right/left sides. Statistical significance level was accepted as $\alpha=0.05$.

RESULTS

The mean age of the 9 fixed cadavers used in the study was $67.56 (\pm 8.443)$ years. The number of units, median and quartile values of the variables measured for the morphometry of the MM are given in **Table 1** and **Table 2**. There was a statistically significant difference between the sexes in the morphometric measurements of the MM A-F, B-C, B-D, B-G, E-D, F-E (**Table 1**). When the measurements of the muscle were compared between the right and left sides, a statistically significant difference was found only in the E-D measurement (**Table 2**).

The thickness values of the MM are presented as medians in **Table 3**. The median value of the muscle thickness was $6.47 (6.06-6.50)$ mm. There was no statistically significant difference in muscle thickness between the right and left sides and between sexes ($p>0.05$). The vertical distance from the midpoint of the distance between the tragus and the lateral canthus to the nerve (point b) was $42.72 (39.09-44.50)$ mm (**Table 4**). There was no statistically significant difference between sexes and between right and left sides ($p>0.05$).

DISCUSSION

The MM and MN are important anatomical structures for many applications such as BTX-A, facial reanimation and acupuncture. In this study, morphometric measurements of the MM and the topographic location of the MN were presented. A statistically significant difference was found between the sexes in the measurements expressing the length and width of the MM ($p<0.05$). Additionally, the study determined that a vertical line descending from the midpoint of the distance between the tragus and the lateral canthus reaches the branching point of the MN. There was no statistically significant difference in this measurement, which reports the topographic location of the nerve, according to sex and side ($p>0.05$). Thus, the measurement point determined for the nerve can be used regardless of sex and side.

The MM, which consists of three parts—superficial, middle, and deep—was found to have smaller dimensions in women ($p<0.05$). This difference may be attributed to variations in the mastication process based on sex. Previous studies have reported that men exhibit a higher chewing frequency and shorter chewing duration compared to women.^{12,13} Furthermore, we believe that the statistically significant difference observed in only one variable between the right and left sides in morphometric measurements of the muscle does not affect the overall results of our measurements.

In the literature, a study by Lee et al.¹⁴ reported the width of the superficial part of the MM as 33.3 ± 4.4 mm, the width of the deep part as 18.5 ± 4.0 mm, the anterior length of the muscle as 64 ± 5.4 mm, and the posterior length as 49.9 ± 4.6 mm. Recently, muscle thickness has been considered an indicator of masticatory muscle function. Muscle thickness is associated with various factors, including bone morphology and physical activity.¹⁵ A direct relationship has been observed between MM thickness and different skeletal structures: the muscle is thicker in individuals with short faces and thinner in those with long faces.¹⁶

In the literature, MM thickness has been measured using various radiological imaging methods.^{15,17,18} Differences in measured muscle thickness exist among imaging methods. Studies have shown that the relaxed muscle thickness measured using ultrasonography is smaller than that measured with MRI.¹⁸ Ultrasonography is considered an accessible, reliable, and practical tool among imaging methods in clinical settings. However, measurement errors can occur due to the position of the probe or excessive pressure applied to the skin.¹⁹ Standardization of methods and parameters is required to prevent measurement errors.

In a study by Rani et al.¹⁵, using ultrasonography, men were found to have a thicker MM. In the present study, no significant difference in MM thickness was found between sexes. Additionally, smaller muscle thickness values were observed compared to the study by Rani et al.¹⁵ Considering that formaldehyde causes tissue shrinkage, the differences in findings between the two studies may be due to the measurement methods used.

There are multiple treatment options available for MMH. To avoid postoperative complications, BTX-A injections are

Table 1. Comparison of morphometric measurement values of MM between sexes

Parameters	Total (n=9) median (25.Q-75.Q) (mm)	Sex		p*
		Female (n=4) median (25.Q-75.Q) (mm)	Male (n=5) median (25.Q-75.Q) (mm)	
A-B	32.55 (29.74-34.68)	32.55 (31.60-33.39)	32.92 (27.20-35.78)	0.897
A-F	65.42 (61.47-65.91)	61.47 (60.27-62.39)	65.76 (65.44-67.74)	<0.001***
B-C	18.56 (16.36-19.31)	16.46 (15.40-18.57)	18.97 (18.34-21.03)	0.027***
B-D	49.23 (46.12-51.78)	45.98 (44.69-51.81)	49.56 (49.16-51.97)	0.034***
B-G	26.87 (22.49-27.10)	23.23 (22.26-26.42)	27.03 (25.83-27.58)	0.016***
C-G	21.46 (19.77-22.26)	19.52 (16.42-23.33)	21.46 (20.99-21.71)	0.573
E-D	22.21 (18.89-23.51)	23.21 (22.33-23.90)	19.33 (17.78-20.59)	0.006***
F-E	23.88 (22.87-31.95)	22.74 (21.60-23.70)	31.63 (23.88-32.61)	0.001***

A-B: Upper edge length of the superficial part of the MM, A-F: Anterior edge length of the superficial part of the MM, B-C: Superficial upper edge of the deep part of the MM, B-D: Posterior edge length of the superficial part of the MM, B-G: Superficial anterior edge of the deep part of the MM, C-G: Posterior edge length of the deep part of the MM, E-D: Lower posterior edge length of the superficial part of the MM, F-E: Lower anterior edge length of the superficial part of the MM, MM: Masseter muscle, p*: Comparison between sexes (Wilcoxon test). ***p<0.05 there is a statistically significant difference

Table 2. Comparison of morphometric measurement values of MM between sides

Parameters	Total (n=9) median (25.Q-75.Q) (mm)	Side		p*
		Right (n=9) median (25.Q-75.Q) (mm)	Left (n=9) median (25.Q-75.Q) (mm)	
A-B	32.55 (29.74-34.68)	33.31 (28.30-34.57)	32.20 (29.52-34.72)	0.514
A-F	65.42 (61.47-65.91)	65.45 (61.06-66.03)	65.42 (61.82-66.47)	0.440
B-C	18.56 (16.36-19.31)	18.97 (16.13-19.09)	18.56 (16.23-19.88)	0.260
B-D	49.23 (46.12-51.78)	49.16 (45.54-52.56)	49.56 (46.24-51.83)	0.678
B-G	26.87 (22.49-27.10)	26.87 (22.59-27.17)	26.91 (22.49-27.10)	0.906
C-G	21.46 (19.77-22.26)	21.29 (19.10-21.87)	21.46 (19.11-22.47)	0.314
E-D	22.21 (18.89-23.51)	22.13 (18.23-23.24)	22.47 (18.71-23.72)	0.021***
F-E	23.88 (22.87-31.95)	23.59 (22.67-32.23)	24.62 (22.98-32.13)	0.373

A-B: Upper edge length of the superficial part of the MM, A-F: Anterior edge length of the superficial part of the MM, B-C: Superficial upper edge of the deep part of the MM, B-D: Posterior edge length of the superficial part of the MM, B-G: Superficial anterior edge of the deep part of the MM, C-G: Posterior edge length of the deep part of the MM, E-D: Lower posterior edge length of the superficial part of the MM, F-E: Lower anterior edge length of the superficial part of the MM, MM: Masseter muscle, p*: Comparison between sides (Wilcoxon test). ***p<0.05 there is a statistically significant difference

Table 3. Comparison of MM thickness by sex and side (mm)

Parameters	Total (n=9) median (25.Q-75.Q) (mm)	Sex		p*	Side		p**
		Female (n=4) median (25.Q-75.Q) (mm)	Male (n=5) median (25.Q-75.Q) (mm)		Right (n=9) median (25.Q-75.Q) (mm)	Left (n=9) median (25.Q-75.Q) (mm)	
Muscle thickness	6.47 (6.06-6.50)	6.48 (6.47-6.67)	6.09 (5.63-6.48)	0.055	6.47 (6.25-6.63)	6.47 (5.44-6.48)	0.400

MM: Masseter muscle, p*: Comparison between sexes, (Mann-Whitney test), p**: Comparison between parties, (Wilcoxon test)

Table 4. The distance between the tragus and the lateral canthus (a) and the vertical distance from the midpoint of this line to the branching point of the MN (a-b distance) by sex and side (mm)

Parameters	Total (n=9) median (25.Q-75.Q) (mm)	Sex		p*	Side		p**
		Female (n=4) median (25.Q-75.Q) (mm)	Male (n=5) median (25.Q-75.Q) (mm)		Right (n=9) median (25.Q-75.Q) (mm)	Left (n=9) median (25.Q-75.Q) (mm)	
Distance between tragus and lateral canthus	76.04 (72.36-79.02)	72.29 (71.55-78.71)	76.56 (75.85-79.02)	0.083	75.85 (72.67-79.59)	76.56 (72.29-78.75)	0.767
a-b distance	42.72 (39.09-44.50)	42.94 (42.70-45.93)	40.71 (34.78-44.80)	0.101	42.89 (37.29-45.22)	42.68 (37.98-44.81)	0.483

MN: Masseteric nerve, b: Branching point of the MN, p*: Comparison between sexes, (Mann-Whitney test), p**: Comparison between sides, (Wilcoxon test)

frequently used as an alternative to surgical operations.¹² Since 1994, intramuscular BTX-A injections have become the standard non-surgical method for treating MMH. Although it is the standard method, there is no consensus on the most effective injection technique. Some studies recommend using single or low-point injection techniques instead of multi-point injections due to their reduced pain and faster administration.²⁰

The optimal dosage of BTX-A varies due to differences in variables, ethnic backgrounds, or relevant morphometric data in studies.²¹ Anatomical studies of the region can contribute

to the literature by aiding in the determination of effective injection techniques and the appropriate number of injection points.

Botulinum toxin-A (BTX-A) is considered a reliable method for the treatment of MMH; however, complications such as bruising, swelling, and muscle weakness may occur if the injection is administered to the wrong area.²² Preventing such complications requires a detailed understanding of the MM and the surrounding anatomical structures. In the literature, various anatomical landmarks and entry points have been described for BTX-A injection.

Kim and colleagues²³ proposed a method involving two points along a line drawn between the tragus and the angle of the mouth (chelion), along with two additional points located 1 cm above and below this line. Additionally, injections performed at 1 cm intervals in the lower third of the muscle are also commonly used techniques.²⁴ Anatomical landmarks described in the literature for identifying the motor nerve of the MM serve as crucial guides for determining injection reference points for BTX-A. Furthermore, the diversity of available techniques provides clinicians with the flexibility to choose the method that best suits their needs and preferences.

On the other hand, the MN is utilized in facial reanimation procedures.^{5,25} Facial reanimation is a challenging field, with various nerve transfer options available, such as the hypoglossal nerve, the contralateral facial nerve, and the MN.⁵ Studies suggest that the MN is a more advantageous option compared to other nerves due to factors such as its anatomical location, relative reliability, strong motor impulses, and low morbidity.¹¹ Additionally, the literature includes evidence that combining multiple nerve transfers can provide greater benefits.^{26,27}

There are studies regarding the location of the MN, which innervates the MM. Previous research has provided guidance for identifying the location of this nerve.^{28,29} Cotrufo et al.²⁸ defined the masseteric region using the mandibular notch and the zygomatic arch to locate the MN. Additionally, Kaya et al.³⁰ measured the distance between the tragus and lateral canthus as 8.4 ± 1.8 cm, while Ganapathy et al.³ reported the preauricular to lateral canthus distance as 7.25 cm on the right and 6.95 cm on the left. The measurements in this study yielded similar results. Thus, the anatomical landmarks used for the nerve are consistent with the literature, reliable, and applicable.

Using two standard anatomical landmarks—the tragus and the lateral canthus—the branching point of the MN was identified. This allowed for determining the topographic location of the MN without requiring any imaging device. These standard anatomical landmarks are easily identifiable points, enabling the motor point of the MM to be located on the skin without any prior preparation.

Limitations

The limitation of this study is the low number of cadavers. Future studies could examine the relationship of the MN with nearby nerve branches and conduct clinical investigations for a more detailed analysis of the MN.

CONCLUSION

Knowing the topographic location of the MN is crucial for accurately performing interventions such as facial reanimation and BTX-A injections. In this study, the method used to locate the MN through the skin is considered to be highly repeatable. The study proposes a repeatable, reliable, and easily understandable location for identifying the nerve without requiring prior preparation.

ETHICAL DECLARATIONS

Ethics Committee Approval

Approval for this study was obtained from Kocaeli University Faculty of Medicine Non-interventional Clinical Researches Ethics Committee (Date: 19.12.2024, Decision No: GOKAEK 2024-/21.01).

Informed Consent

This study was conducted using human cadaveric material provided by the anatomy laboratory of Kocaeli University Medicine Faculty, in accordance with ethical guidelines and institutional regulations.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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